REMARKS

Applicants respectfully request reconsideration and withdrawal of the rejections set forth in the Office Action. Claims 1-17 are pending in the application. Claims 9-12 and 16 are withdrawn from consideration. Claims 1-8, 13-15 and 17 stand rejected. New claims 18-37 are proffered to replace claims 1-8, 13-15 and 17. Claims 18-37 are pending.

Applicants' new claims recited an antigen preparation prepared by the methodology disclosed in the application, in which proteins of particular molecular weights are employed. Claim 18 is supported by original claim 1 and in the specification, for example, on page 13 lines 12-36. Claim 19 is supported by previously filed claim 12. Claim 20 is supported by previously filed claim 2. Claims 21 and 22 are supported by previously filed claim 3. Claims 23, 24, 25, 28, and 29 are supported in the specification, for instance, on page 12 lines 11-25. Claims 26, 30 and 31 are supported by previously filed claim 6. Claims 32, 33 and 34 are supported in the previously filed claim 7. Claims 35 and 36 are supported in previously filed claims 14 and 15, respectively.

Rejection under 35 USC § 112 second paragraph

The examiner rejected claim 1, alleging the omission of essential steps. Claim 18 has been revised to clarify when the antigen is added to the sample and how the antibody levels are determined.

The examiner rejected the form of claims 6, 7, and 17 for improper recitation of a Markush group. Applicants believe that new claims 30-33 are not subject to this criticism.

Rejection under 35 USC § 102(b)

Claims 1-8 and 17 were rejected for alleged anticipation by Buckley *et al.* (US 4,806,465). The Buckley reference discloses purified cytoplasmic antigens that are defined by their reactivity with two monoclonal antibodies, produced by the hybridomas ATCC HB-8397 and ATCC HB-8398. Applicants have determined, however, that there is no reactivity between the claimed *Candida* antigen preparation and either of these antibodies, measured via

immunoblotting or ELISA. This information can be provided. However, applicants believe that a declaration is unnecessary because the antigens described in Buckley and the antigens claimed in this application are extracted from different forms of *Candida* and, hence, are unlikely to be the same.

More particularly, the Buckley reference describes antigens extracted from the mycelial form of *Candida*, whereas applicants' recited antigen is prepared from the blastospore form. The phenotypic switching displayed by *Candida* involves the regulation of phase-specific genes. This switching results in antigenic variability on the yeast cell surface, because different genes are activated in different forms. Because the different forms of *Candida* have different active genes, resulting in antigenic variability, the skilled person would expect the antigens extracted from these different forms to be dissimilar directly contrary to the examiner's premise. Therefore, the Buckley reference, teaching antigens extracted from the mycelial form of *Candida,* does not anticipate the present invention, which involves antigens prepared from the blastospore form of *Candida*.

Additionally, Buckley describes antigens with a molecular weight of 48-52 kDa, while applicants' claimed invention covers antigens having a molecular weight of 55 kDa, 30 kDa and 20 kDa. Because the molecular weights of the disclosed antigens are different, the antigens themselves must be different.

Rejections under 35 USC § 103(a)

The examiner rejected claims 13 and 14-15 over Buckley et al. (US 4,806,465) in view of Ballentyne et al., *Biotechnology and Applied Biochemistry*, Vol. 31, pages 213-218 (2000), and Miyada et al. (US 5,766,874), respectively. Applicants have demonstrated already that the Buckley reference describes antigens, derived from the mycelial form of *Candida*, that are defined by their reactivity to two monoclonal antibodies produced by hybridomas, ATCC #HB-8397 and 8398. Because applicants' claimed antigen preparation fails to react with these same monoclonal antibodies, the examiner's reliance on the Buckley reference is unavailing. Further, because the claimed antigen preparation is derived from the Blastospore form of *Candida*, one of ordinary skill would not have considered Buckley to

implicate the presently claimed antigen preparation. Accordingly, the combination of Buckley with Ballentyne et al. or Miyada et al. does not establish a *prima facie* case of obviousness.

CONCLUSION

Based on the foregoing, applicants submit that the present application now is in condition for allowance, and a timely indication to this effect is respectfully requested. Examiner Shahnan Shah is invited to contact the undersigned directly, should he feel that a telephone interview would advance the prosecution of this application.

Respectfully submitted,

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

Marked Up Version Showing Changes Made in the Specification

On page 10, please replace lines 5-20 with

--- Chromophoric molecules that may be used are 2,3-dinitrobenzene (DNB) salts, dinitrophenol (DNP) and methyl and butyl orange. Other suitable chromophoric agents are well known in the art. Enzochromic molecules that may be conjugated with the antibody are enzymes that give colour with appropriate reagents. Examples are alkaline phosphatase (ALP) which develops colour with nitrophenyl phosphate (NPP), glucose oxidase with glucose, and D-galactopyranoside. These and other examples are well known in the art. Examples of dinitrofluorobenzene and "pipsyl" derivatives. Luminogenic molecules may be conjugated to antibodies by the method of Branchini, et al. (Biochem. Biophys. Res. Commun. 97, 334 (1980)). The term "chromophoric" hereinafter is intended to include "enzochromic", "fluorochromic" and "luminogenic" molecules as well.---

On page 18 please replace lines 18-22 with the following:

--- A clinical isolate of the *Candida albicans*, was obtained from a patient with vaginal thrush. The identity of the *Candida* species was confirmed with the use of an API® 20C Auxonagram strip (API System S.A., France). The *C. albicans* isolate was designated KEMH5.---

On page 19 please replace lines 3-11 with the following:

--- *Candida* cells were ruptured mechanically with the use of a the use of a DYNOMILL™ (WAB, Switzerland). Milling was continued until 99% cell disruption was obtained. The soluble *Candida* cell extracts were collected and dispensed into 50ml centrifuge tubes. The extracts were centrifuged for 12h at 8,517 x g and 4°C to precipitate insoluble cell walls. The supernatants containing the soluble cytoplasmic antigen fraction were recovered and passed through a 0.45μm filter membrane.---

Please replace the paragraph bridging pages 19 and 20 with the following:

--- The soluble cytoplasmic antigen fraction was dialysed overnight against 20mM Tris.Cl, pH7.4. An estimate of the quantity of protein in solution was performed using the [Bio-rad®] **BIO-RAD®** (Bradford) microassay procedure in accordance with the manufacturers instructions. A portion of the cytoplasmic antigen extract was analysed by SDS-PAGE. ---

On page 20 please replace lines 10-26 with the following:

--- Purification of the enolase antigen was conducted in the same fashion as the soluble *Candida* cytoplasmic antigen except that it was not subjected to Con A-Sepharose chromatography. Instead, following dialysis and filtering through a 0.20µm syringe filter (cellulose acetate), the filtered extracts were applied to a Pharmacia Biotech XK 50/20 chromatography column packed with Pharmacia Biotech Source 15Q quaternary ammonium anion exchanger (Pharmacia LKB, Uppsala, Sweden). The column was equilibrated prior to chromatography with column binding buffer 'A' (20mM bis-Tris, pH 6.5). Anion exchange chromatography of the crude extracts was controlled and recorded using the [Bio-Rad] **BIO-RAD®** and [Econo®] **ECONO®** system (Bio-Rad Laboratories, USA). Bound protein was eluted from the column with a salt gradient of buffer 'B' (1M NaCl in buffer 'A', pH 6.5). The recovered fractionated proteins were analysed by an enzyme activity assay. ---

Please replace the paragraph bridging pages 20 and 21 with the following:

--- The active enzyme enolase hydrolyses D(+)2-phosphoglyceric acid (PGA) to phosphoenolpyruvate (PEP). The production of PEP can be monitored by spectrophotometry at 240nm. 20µl of protein solution was combined with 1ml of enolase substrate solution (50mM Tris-HCl pH 7.4, 2.7mM magnesium acetate, 1.0mM EDTA, 1.2mM D(+)2-phosphoglyceric acid) in a quartz cuvette and the change of absorbance recorded at 1min intervals. The specific activity was defined as the conversion of 1µmol of PGA to PEP per mm per mg protein. An estimate of the quantity of protein in solution was performed using the [Bio-Rad®] **BIO-RAD®** (Bradford) microassay procedure. ---

On page 21 please replace lines 10-26 with the following:

--- Eluate fractions containing enolase activity were selected and dialysed for 12h at 25°C in hpH_2O. The dialysed fractions were recovered and filtered through a 0.20µm syringe filter. The filtrate was concentrated ten-fold by evaporation under vacuum for 5h. The concentrated samples were dialysed with binding buffer 'A' (10mM sodium acetate, pH 4.7) immediately prior to application to a Pharmacia Biotech Mono S HR10/10 chromatography column packed with methyl sulphonate cation exchanger (Pharmacia LKB, Uppsala, Sweden). Cation exchange chromatography performed using the [Bio-Rad®] **BIO-RAD®** Biologic system. Bound protein fractions were eluted from the column with a salt gradient of buffer 'B' (1M NaCl in buffer 'A', pH 4.7). Fractions containing enolase activity were identified by the enzyme activity assay described above. ---